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(54) Title: IM PEPTIDES

(57) Abstract

The invention is directed to the diagnosis and treatment of herpes virus related diseases. By noting within the Epstein-Barr virus (EBV) open reading frames (ORFs) transcribed late in the viral cycle for which a translation product may or may not have been established, and synthesizing one or more polypeptides each of which includes at least one segment, each segment comprising at least part of the amino acid sequence identified in that ORF, a specific and reliable diagnostic test for, and treatment of, infectious mononucleosis and related diseases is possible. The preferred amino acid sequences for these segments include NSPKNG, KNGSNQ, SNQLVI, AHARDK, RDKAGA, VMAMIL, SEPRPR and PSRTPS. These sequences can be further combined to provide polypeptides with at least one segment comprising sequences selected from AHARDKAGAVMAMIL, ASLNSPKNGSNQLVI, ELESEPRPRPSRTPS, QAMKKIEDKVRKSVD, SRSRGREAKKVQISD, LIKASLRKDRKLYAE, VSFSKTRRAIRESRA, CNYSAGEEDDQYHAN, RPHRRPVSKRPTHKP, EITQEENRGEQRLGH, GALRARLDRPRPTAQ, NSPKNGSNQAHARDKSEPRPR, NSPKNGSNQRDKAGASEPRPR, NSPKNGSNQSEPRPRKNGSNQ, NSPKNGSNQLVISEPRPRPSRTPS, NSPKNGSNQLVIPSRTPS and NSPKNGSNQAHARDKAGASEPRPR.

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TITLE: IM PEPTIDES

TECHNICAL FIELD

THIS INVENTION is directed to the diagnosis and treatment of herpes virus related diseases. In particular, it relates to the use of specific open reading frames (ORFs) within the Epstein-Barr virus (EBV) which encode antigens recognized by EBV-specific antibodies raised during infectious mononucleosis (IM) and related diseases and the use of synthetic peptides based on the amino acid sequences encoded by these ORFs in a specific and reliable diagnostic test for, and treatment of, IM and related diseases.

BACKGROUND ART

Epstein-Barr virus (EBV) is a member of the herpes virus family and is present in all human populations. Primary infection usually occurs in early childhood and remains silent throughout a person's life. However, when uninfected adolescents and young adults are exposed to EBV, about 60% manifest infectious mononucleosis (IM).

The predominant laboratory test used to establish the diagnosis of IM has been the demonstration of heterophil antibodies. The rapid slide tests have become the most widely used method to detect these heterophil antibodies. In contrast, quantitative agglutination tests, such as the Paul-Bunnell-Davidsohn method, are more accurate but are also more tedious and time consuming.

The use of tests to measure heterophil antibodies have limitations, namely, only between 80-95% of IM patients produce these antibodies, these antibodies are absent in

a large percentage of young children and the antibodies are produced in a variety of other diseases such as lymphoma, hepatitis and leukemia. The measurement of heterophil antibodies also does not give any indication of the severity of the disease and cannot be used to monitor the course of IM.

Immunofluoresence tests that measure antibodies to EBV can also be used in the diagnosis of heterophil-negative cases of IM, or patients with atypical manifestations.

These tests, however, are time consuming and require the use of trained personnel and specialized equipment which does not make them amenable to the routine analysis of large numbers of samples.

The diagnosis of an acute primary EBV infection can also be determined by an IgM response to EBV-viral capsid antigen (VCA). The VCA is composed of a large number of different antigens. Components of VCA are defined by the fact that they are expressed late in the replicative cycle of the virus. Many VCA components have been mapped to specific open reading frames (ORF's) within the EBV genome though there are many ORF's, known to be expressed late in replication, to which specific VCA antigens have not yet been identified.

Genetic engineering and synthetic polypeptide technologies now enable the manufacture of large quantities of protein and polypeptide antigens. However, these techniques are only effective if the amino acid residue sequence of the native protein is known.

The amino acid residue sequence of a natural protein can be determined by sequencing of the protein itself.

Alternatively, the DNA sequence that codes for the protein may also reveal the protein's amino acid residue sequence.

Antibodies can be used to determine whether an ORF present in a DNA sequence codes for a protein. This involves manufacturing an array of protein fragments or synthetic polypeptides whose amino acid residue sequences correspond to the hypothetical sequences obtained from the ORFs. The protein fragments or polypeptides to which naturally occurring antibodies immunoreact thereby identify the ORF as encoding a naturally occurring protein. The complete amino acid sequence of this protein could then be deduced from the DNA sequence of the ORF.

DISCLOSURE OF THE INVENTION

It is a general object of the present invention to overcome, or at least ameliorate, one or more of the above disadvantages, and to provide a specific and reliable test for the diagnosis for, and treatment of, IM and related diseases.

As the complete DNA sequence for EBV has been identified, including the start and stop codons which, prima facie, define potential ORFs for the transcription of the genetic code, the present inventors have synthesized peptides based on the predicted amino acid sequences encoded by these ORFs, even though the proteins to which these ORFs may relate were not first established as being produced by the virus, and have

demonstrated that EBV-specific antibodies raised during IM react with the synthesized peptides.

Thus, according to a first aspect of the present invention, there is provided a peptide comprising a sequence which includes at least one segment which codes for an antigen recognized by EBV-specific antibodies raised during IM or a related disease.

As a second aspect, the present invention also includes within its scope a method of identifying a polypeptide suitable for use in the diagnosis of IM and related diseases, said method comprising:

(1) noting within EBV open reading frames transcribed late in the viral cycle for which a translation product may or may not have been established;

(2) synthesising one or more polypeptides each of which includes at least one segment wherein each segment comprises at least part of the amino acid sequence identified in that open reading frame; and

(3) determining whether said polypeptide is effective in the diagnosis of IM and related diseases.

When sera from patients who exhibited clinical symptoms of IM and related diseases were assayed using the peptides of the invention, a positive reaction was noted with a high correlation between this assay and known assays for identifying IM and related diseases.

Therefore, according to a third aspect of the present invention, there is provided a method of diagnosis of infectious mononucleosis or a related disease, said method comprising assaying serum from a patient suspected of having infectious mononucleosis or a related disease with at least one peptide as hereinbefore defined.

The present invention also provides, as a fourth aspect, a kit for use in the diagnosis of IM or a related disease, said kit comprising:

(a) at least one peptide as hereinbefore defined; and

(b) a means for indicating the presence of a reaction, particularly an immunoreaction, between said at least one peptide and another molecule(s), especially anti-EBV antibodies.

Since a patient with IM or a related disease contains antibodies to the peptides of the invention, it is likely that the peptides of the invention, when administered to the patient, would elicit anti-EBV antibodies.

Therefore, as a fifth aspect of the present invention, there is provided a vaccine that, when administered, is capable of inducing antibodies effective against EBV, said vaccine comprising:

(a) at least one peptide as hereinbefore defined; and

(b) a carrier and/or diluent and/or adjuvant.

As used throughout the specification, the term "carrier or diluent" denotes an organic or inorganic, natural or synthetic material with which the active ingredient is combined in order to facilitate the administration of the vaccine of the invention. This carrier or diluent is, therefore, generally inert and it must be pharmaceutically acceptable. Similarly, the term "adjuvant" has the usual meaning in the art to describe a material which aids the operation of the active ingredient.

According to a sixth aspect of the present invention, there are also provided antibodies and substantially whole antibodies raised to - or induced by - the peptides of the invention as hereinbefore defined.

These molecules are collectively referred to as receptors and can be raised in animal hosts using the vaccine as hereinbefore defined.

Preferably, the peptide of the invention comprises at least one segment selected from the following sequences:

AHARDKAGAVMAMIL
ASLNSPKNGSNQLVI
ELESEPRPRPSRTPS
QAMKKIEDKVRKSVD
SRSRGREAKKVQISD
LIKASLRKDRKLYAE
VSFSKTRRAIRESRA
CNYSAGEEDDQYHAN
RPHRRPVSKRPTHKP
EITQEENRGEQRLGH
GALRARLDRPRPTAQ

More preferably, the peptide of the invention comprises at least one segment selected from the following sequences:

NSPKNG
KNGSNQ
SNQLVI
AHARDK
RDKAGA
VMAMIL
SEPRPR
PSRTPS

Most preferably, the peptide of the invention comprises at least one segment selected from the following sequences:

NSPKNGSNQAHARDKSEPRPR
NSPKNGSNQRDKAGASEPRPR
NSPKNGSNQSEPRPRKNGSNQ
NSPKNGSNQLVISEPRPRPSRTPS
NSPKNGSNQLVIPSRTPS
NSPKNGSNQAHARDKAGASEPRPR

A particularly preferred peptide of the invention comprises at least one segment containing the sequence:

NSPKNGSNQLVIPSRTPS

All amino acid residues identified throughout the specification are in the natural or L-configuration. In

keeping with standard polypeptide nomenclature, abbreviations for amino acid residues are as follows:

SYMBOL	AMINO ACID
Y	L-tyrosine
G	glycine
F	L-phenylalanine
M	L-methionine
A	L-alanine
S	L-serine
I	L-isoleucine
L	L-leucine
T	L-threonine
V	L-valine
P	L-proline
K	L-lysine
H	L-histidine
Q	L-glutamine
E	L-glutamic acid
W	L-tryptophan
R	L-arginine
D	L-aspartic acid
N	L-asparagine
C	L-cysteine

DETAILED DESCRIPTION OF EMBODIMENTS

SUBJECTS, MATERIALS AND METHODS

Preparation And Use Of Synthetic Peptides

Peptides (15 aa each) were synthesized by the Multiple Simultaneous Peptide technique (MSPS) of Houghton, R.A. (1985, Proc. Natl. Acad. Sci. USA 82, 5131-5135). The synthetic peptides were linked to bovine serum albumin (BSA) with glutaraldehyde as described by Bulinski et

al. (1983, Proc. Natl. Acad. Sci. USA **80**, 1506-1510). Essentially, 5mg of dry peptide were added to 0.5ml (4mg/ml) BSA in 100mM phosphate buffer, pH 7.3. To this were added 0.25ml of 0.25% (v/v) glutaraldehyde for each mg of dry peptide. The solution was left in the dark over night at 21°C to conjugate, after which the solution was extensively dialyzed against PBS containing 50mM glycine, pH 7.3. The conjugated peptides were then stored at -20°C until needed. Peptide sequences, deduced from 13 different ORFs transcribed late in viral replication, were synthesized. These peptides are identified in Table 1.

TABLE 1

VCA PEPTIDES

ORF	PEPTIDES	NUMBER
BCRF1	AENQDPEAKDHVNSL	1
BCRF1	FFQTKDEVDNLLLKE	7
BDLF2	AKAEERTAEMDDTMA	3
BDLF2	GGMKRKQCRVDRLTD	8
BDLF3	AHARDKAGAVMAMIL	2
BDLF3	PTVPDERQPSLSYGL	12
BKRF2	ASLNSPKNGSNQLVI	4
BILF2	CNYSAGEEDDQYHAN	20
BILF2	RPHRRPVSKRPTHKP	23
BSRF1	EEPETFECPDRWRAE	5
BGLF1	EITQEENRGEQRLGH	21
BGLF1	VSFSKTRRAIRESRA	18
BALF1	LIKASLRKDRKLYAE	9
BALF1	YAVFTRDEKDLPLPA	19
BBRF3	ELESEPRPRPSRTPS	6
BBRF3	RSSTSSSSSRSTRRQ	15
BXRF1	GALRARLDRPRPTAQ	22
BXRF1	PRSARAGRAGGRKGQ	11
BORF1	MKVQGSVDRRRLQRR	10
BORF1	RGSEFTRDVRGLVEE	14
BLRF2	QAMKKIEDKVRKSVD	13
BLRF2	SRSRGREAKKVQISD	16
BMRF2	TSGLERRRSIFCARG	17

ELISA assay

The stock peptide-BSA conjugate was diluted 1/100 with DD H_2O and 50 µl of the peptide-BSA conjugate were added to each well in 96 well microtiter plates (Flow Laboratories) and the plates were left overnight at 37°C to dry. To block the wells 200µl of a solution containing 5% BSA, 0.5M carbonate buffer pH 9.0 were added and left for 30 minutes at ambient temperature. The wells were then washed four times with 200 µl of 0.1% BSA-PBS/1%Tween. Human sera (diluted 1 in 100 in 5%BSA/2xPBS/1%Tween) were added and incubated at 21°C for 1h. The plates were then washed six times with 0.1%BSA/2xPBS/0.1%Tween and 100 µl of peroxidase-labelled anti-human IgM (Tago, µ fraction) (diluted 1/5000 in 5%BSA/2xPBS/0.1%Tween) were added and then the plates incubated at 37°C for 30 minutes. The plates were again washed six times as above. The plates were given a rinse with distilled water (neutral pH), and substrate (100 µl of 1mM ABTS (2,2-azino-bis(3-ethlybenzthiazoline-6-sulphonic acid) diammonium salt (Sigma, St. Louis, USA) in 100mM phosphate-citrate buffer (pH 4.3) containing 0.004% (v/v) hydrogen peroxide) were added and the plates incubated at 37°C for 30 minutes. Finally the plates were read at 410nm.

Subjects and sera

Samples of sera (26) were obtained from patients with IM. These patients were diagnosed as having clinical symptoms of IM and were confirmed by immunofluoresence assays for IgM and IgG antibodies to VCA and by immunoblotting for antibodies to early antigens (EA) and absence of antibodies to EBV nuclear antigen 1 (EBNA1). Sera (14 were EBV seropositive and 8 EBV seronegative) were also obtained from healthy controls and their EBV

status was determined by immunofluoresence assays to the EBV antigens. The normal controls were all negative for IgM antibodies to EBV. For the clinical trials, hundreds of samples of serum, from patients displaying IM-like symptoms, were collected.

Immunofluoresence assay for EBV antigens.

Anti-VCA titres were measured according to the method of Henle and Henle. (J Bacteriol. 91, 1248-1256).

Heterophil antibody assay

The Paul Bunnell test was used to measure heterophil antibody titres in serum.

RESULTS AND DISCUSSION

Screening of EBV peptides

The 23 synthetic peptides were initially screened against 26 samples of sera from confirmed IM patients, 8 EBV seronegative controls and 14 EBV seropositive controls. The results, presented in Fig. 1, illustrate that a number of peptides were reactive with IgM antibodies from IM patients while showing little reaction with the sera from either EBV seropositive or seronegative controls. Peptides # 2, 4, 6, 13, 16 and 18 were selected for further studies.

Reaction of IM and normal sera with selected EBV peptides.

Comparison of total Ig and IgM reactions with the peptides indicated that normal seropositive individuals lacked both IgM and IgG antibodies to these peptides

(Fig. 2). However, it appeared that IM patients contained only IgM antibodies to these peptides. Measurement of IgG antibodies to the peptides, in both IM sera and sera from normal controls, confirmed the absence of these antibodies (results not shown).

These results indicated that measurement of either IgM or total Ig could be useful in identifying serum samples from IM patients.

Since only IgM antibodies to the peptides appear to be present in IM patients, there should not be a problem with rheumatoid factor or interference from IgG antibodies, both of which are usually a problem with indirect ELISA assays.

Clinical study

In order to ascertain the viability of using the peptides to identify cases of IM, sera were obtained from patients who showed clinical symptoms of IM. These sera were assayed by ELISA, using the 6 selected synthetic peptides, by immunofluoresence for the presence of IgM antibodies to VCA and for heterophil antibodies. Patients were considered to have IM if they were both heterophil positive (titres of 1/16 or higher) and IgM positive (titres of 1/40 or higher) by immunofluorescence or heterophil negative but with atypical monnuclear cells and IgM positive by immunofluoresence. The ELISA based IgM assay using the synthetic peptides was considered to be positive when a serum reacted with four or more of the peptides (A^{410} of 0.40 or above). The results of the assays, obtained with all of the patients, are shown in Table 2.

TABLE 2

Patient	Heterophil	ATLs (%)	ELISA (IgM)	IFA (IgM)
1	<10	0	-	<10
2	64	13	+	160
3	<10	0	-	<10
4	<10	0	-	<10
5	<10	0	-	<10
6	<10	0	-	<10
7	<10	0	-	<10
8	<10	0	-	<10
9	<10	0	+	160
10	<10	0	-	<10
11	<10	0	-	<10
12	<10	0	-	<10
13	<10	0	-	<10
14	<10	0	-	<10
15	<10	0	-	<10
16	<10	0	-	<10
17	<10	0	-	<10
18	<10	0	-	<10
19	<10	0	-	<10
20	<10	0	-	<10
21	<10	0	-	<10
22	<10	15	+	160
23	<10	0	-	<10
24	256	21	+	160
25	<10	0	-	<10
26	<10	0	-	<10
27	<10	0	-	<10
28	<10	0	-	<10
29	<10	14	-	<10
30	<10	3	-	<10
31	<10	0	-	<10
32	<10	0	-	<10
33	10	0	-	160
34	<10	0	-	<10
35	<10	0	-	<10
36	<10	0	-	<10
37	<10	0	-	<10
38	<10	0	-	<10
39	<10	0	-	<10
40	<10	24	-	<10
41	<10	0	-	<10
42	<10	0	-	<10
43	<10	0	-	<10
44	<10	16	-	<10
45	<10	0	-	<10
46	64	24	+	360
47	<10	0	-	<10
48	16	0	-	<10
49	<10	2	-	<10
50	<10	0	-	<10

TABLE 2 (cont)

Patient	Heterophil	ATLs (%)	ELISA (IgM)	IFA (IgM)
51	<10	0	-	<10
52	<10	0	-	<10
53	<10	3	-	10
54	<10	0	-	<10
55	<10	0	-	<10
56	64	65	-	320
57	<10	0	-	<10
58	<10	0	-	<10
59	<10	0	-	<10
60	<10	0	-	<10
61	<10	0	-	<10
62	<10	1	-	<10
63	16	0	-	<10
64	256	39	+	160
65	<10	9	-	<10
66	<10	0	-	<10
67	<10	0	-	<10
68	<10	0	-	<10
69	<10	0	-	<10
70	<10	0	-	<10
71	<10	0	+	<10
72	256	23	+	320
73	<10	0	-	<10
74	<10	0	-	<10
75	<10	9	+	40
76	<10	0	-	<10
77	<10	0	-	<10
78	<10	0	-	<10
79	<10	0	-	<10
80	<10	0	-	<10
81	<10	0	-	<10
82	<10	0	-	<10
83	<10	0	-	<10
84	<10	0	-	<10
85	<10	0	-	<10
86	64	19	+	160
87	<10	0	-	<10
88	64	4	+	160
89	<10	0	-	<10
90	<10	0	-	<10
91	64	45	+	160
92	<10	0	-	<10
93	<10	0	-	<10
94	<10	0	-	<10
95	256	32	+	160
96	<10	0	-	<10
97	<10	0	-	<10
98	<10	0	-	<10
99	<10	2	-	<10

TABLE 2 (cont)

Patient	Heterophil	ATLs (%)	ELISA (IgM)	IFA (IgM)
100	<10	3	-	<10
101	<10	0	-	<10
102	<10	0	-	<10
103	<10	0	-	<10
104	16	30	+	160

The results of the statistical analysis of the correlation between the three assays systems are presented in Table 3.

TABLE 3

CORRELATIONS AND POLYCHORIC TEST STATISTICS

	ELISA	IF(IgM)	HETEROPHIL
ELISA	1.000	0.988	0.901
IF(IgM)		1.000	0.964
HETEROPHIL			1.000

ASYMPTOTIC VARIANCES OF ESTIMATED CORRELATIONS

ELISA-IF(IgM)	= 0.00020
ELISA-HETEROPHIL	= 0.00407
IF(IgM)-HETEROPHIL	= 0.00094

The results obtained from the statistical analysis demonstrate a 96% correlation between the IF-IgM assay and the heterophil antibody assay which better the reported data. However, the correlation between the ELISA assay and IF-IgM was 99% indicating that this assay was more accurate at predicting patients with IM. There was one serum which was ELISA positive but IF-IgM negative and it is possible that this patient may have not as yet developed a high enough titre of IgM antibodies to be detected by the IF-IgM test. Two sera samples were positive by IF-IgM and negative by the ELISA assay.

Peptides # 2,4 and 6 were selected for further clinical trials, whereby 510 samples of serum, from patients displaying IM-like symptoms, were assayed for heterophil antibody using the Paul Bunnell test and for the presence of IgM antibodies to EBV by immunofluoresence as described above. These results were then correlated with the results obtained using the ELISA assay with the three synthetic EBV peptides and with the Monolert assay as marketed by Johnson and Johnson. The statistical analysis of these results are shown below.

CORRELATIONS AND TEST STATISTICS (PC=POLYCHORIC)

		TEST OF MODEL			TEST OF ZERO CORR.	
	Correlat.	Chi-squ.	DF	P-VALUE	CHI-SQU.	P-VALUE
IFA VS. PB	.868 (PC)	.000	0	1.000	889.303	.000
ELISA VS. PB	.882 (PC)	.000	0	1.000	969.017	.000
ELISA VS. IFA	.897 (PC)	.000	0	1.000	1073.819	.000
MONO VS. PB	.718 (PC)	.000	0	1.000	413.573	.000
MONO VS. IFA	.595 (PC)	.000	0	1.000	238.506	.000
MONO VS. ELISA	.614 (PC)	.000	0	1.000	259.295	.000

ESTIMATED CORRELATION MATRIX

	PB	IFA	ELISA	MONO
PB	1.000			
IFA	0.868	1.000		
ELISA	0.882	0.897	1.000	
MONO	0.718	0.595	0.614	1.000

PB - Paul Bunnell

IFA - Immunofluoresence assay for EBV-specific IgM

ELISA- ELISA assay using the synthetic EBV peptides of the invention

MONO - Monolert assay as marketed by Johnson and Johnson

These results show a much higher correlation between the synthetic peptides of the invention and the IFA assay than did the PB test or the Monolert assay.

Rather than use three independent peptides in the assay system, longer peptides containing combinations of B-cell epitopes (ie the epitopes to which the antibodies bind) from each of the 15 amino acid peptides were constructed. To define the B-cell epitopes in each of the three peptides overlapping 6 amino acid synthetic peptides were prepared and assayed by ELISA using a pooled IM positive sera and a pooled normal sera.

ORIGINAL 15-mers PEPTIDES

#4 ASLNSPKNGSNQLVI

#2 AHARDKAGAVMAMIL

#6 ELESEPRPRPSRTPS

SYNTHETIC OVERLAPPING PEPTIDES

	PEPTIDE	POSITIVE SERA	NEGATIVE SERA
	ASLNSPKNGSNQLVI		
	ASLNSP	0.287	0.110
**	NSPKNG	0.555	0.143
**	KNGSNQ	0.537	0.141
**	SNQLVI	0.437	0.133
	AHARDKAGAVMAMIL		
**	AHARDK	0.639	0.215
**	RDKAGA	0.496	0.122
	AGAVMA	0.324	0.099
**	VMAMIL	0.505	0.170
	ELESEPRPRPSRTPS		
	ELESEP	0.150	0.063
**	SEPRPR	0.623	0.228
	RPRPSR	0.407	0.131
**	PSRTPS	0.546	0.185

** - INDICATES THE PEPTIDES CONTAINING B-CELL EPITOPES.

Having defined the B-cell epitopes contained within the original 15 aa peptides, different combinations of these epitopes were used in the synthesis of larger synthetic peptides. Examples of some of these combinations are shown below:

EPITOPE COMBINATIONS:

PEPTIDE A.	NSPKNGSNQAHARDKSEPRPR
PEPTIDE B.	NSPKNGSNQRDKAGASEPRPR
PEPTIDE C.	NSPKNGSNQSEPRPRKNGSNQ
PEPTIDE D.	NSPKNGSNQLVISEPRPRPSRTPS
PEPTIDE E.	NSPKNGSNQLVIPSRTPS
PEPTIDE F.	NSPKNGSNQAHARDKAGASEPRPR

These 6 peptides were then tested for their reaction with sera from known IM patients and with sera from normal controls. The sera were all well characterized for the presence/absence of IgM antibodies to EBV by immunofluoresence. The results of that testing are presented in Table 4.

TABLE 4

Peptide	A *	B **	IM1	IM2	IM3	IM4	IM5	NOR1	NOR2	NOR3	NOR4	NOR5	NOR6
A	1.58	0.25	1.34	1.34	1.34	1.52	1.53	0.63	0.16	0.31	0.13	0.25	0.47
B	1.64	0.19	1.20	1.35	1.24	1.63	1.15	0.48	0.19	0.25	0.11	0.20	0.38
C	1.04	0.12	0.81	1.11	0.93	1.47	0.85	0.21	0.15	0.32	0.12	0.14	0.24
D	1.31	0.17	0.97	1.14	1.05	1.49	0.87	0.36	0.16	0.24	0.12	0.14	0.21
E	1.20	0.12	0.64	1.07	0.88	1.46	0.67	0.25	0.15	0.17	0.11	0.13	0.21
F	1.55	0.09	1.15	1.22	1.19	1.60	1.12	0.37	0.21	0.39	0.15	0.24	0.37

* Pooled control IM serum
** Pooled control normal serum
IM Infectious mononucleosis
NOR Normal

These results demonstrate that any of these epitope combinations could be used to identify serum from IM patients. However some of the peptides gave reasonably strong reactions with sera from normal individuals (peptides A,B and F in particular). Of those peptides which had low reactions with the normal sera peptide E gave the lowest values and so was chosen for further studies.

Peptide E was assayed with a larger contingent of sera from patients with IM and normal controls. Control wells, containing only BSA, were also included in the assay to check for sera which may react with the carrier protein. The results of that assay are presented in Table 5.

TABLE 5

ELISA ASSAY OF PEPTIDE E.

SERA	DIAGNOSIS	PEPTIDE WELL	BLANK WELL (BSA)
A	Positive control	0.993	0.066
B	Negative control	0.116	0.068
1	IM	1.050	0.077
2	IM	1.133	0.072
3	IM	0.689	0.056
4	IM	0.780	0.064
5	IM	0.852	0.085
6	IM	0.598	0.051
7	IM	0.813	0.053
8	IM	0.953	0.062
9	IM	1.093	0.051
10	IM	0.739	0.054
11	IM	1.128	0.043
12	IM	0.933	0.056
13	IM	0.795	0.058
14	IM	0.556	0.051
15	IM	0.717	0.047
16	IM	1.454	0.065
17	Normal	0.101	0.049
18	Normal	0.187	0.055
19	Normal	0.098	0.051
20	Normal	0.072	0.052
21	Normal	0.149	0.045
22	Normal	0.061	0.050
23	Normal	0.073	0.044
24	Normal	0.080	0.045
25	Normal	0.120	0.047
26	Normal	0.159	0.052
27	Normal	0.142	0.057
28	Normal	0.054	0.046
29	Normal	0.270	0.076
30	Normal	0.336	0.045
31	Normal	0.280	0.051
32	Normal	0.337	0.041
33	Normal	0.136	0.042

These results demonstrate that peptide E could be used to reliably detect cases of IM while showing little reaction with sera from normal individuals.

The clinical and other data obtained indicates that the ELISA assay using the peptides of the present invention is a specific and reliable test for the diagnosis of infectious mononucleosis and related diseases. The present invention should also find use in the treatment of such diseases.

Those skilled in the art will appreciate that the above embodiments are given by way of exemplification of the invention only, and that changes may be made to the details set out therein without departing from the scope of the invention as defined in the following claims.

CLAIMS

1. A peptide comprising a sequence which includes at least one segment which codes for an antigen recognized by EBV-specific antibodies raised during IM or a related disease.

2. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence AHARDKAGAVMAMIL.

3. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence ASLNSPKNGSNQLVI.

4. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence ELESEPRPRPSRTPS.

5. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence QAMKKIEDKVRKSVD.

6. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence LIKASLRKDRKLYAE.

7. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence VSFSKTRRAIRESRA.

8. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence CNYSAGEEDDQYHAN.

9. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence RPHRRPVSKRPTHKP.

10. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence EITQEENRGEQRLGH.

11. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence GALRARLDRPRPTAQ.

12. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence SRSRGREAKKVQISD.

13. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNG.

14. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence KNGSNQ.

15. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence SNQLVI.

16. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence AHARDK.

17. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence RDKAGA.

18. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence VMAMIL.

19. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence SEPRPR.

20. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence PSRTPS.

21. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQAHARDKSEPRPR.

22. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQRDKAGASEPRPR.

23. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQSEPRPRKNGSNQ.

24. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQLVISEPRPRPSRTPS.

25. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQLVIPSRTPS.

26. A peptide as defined in Claim 1, wherein said at least one segment comprises the sequence NSPKNGSNQAHARDKAGASEPRPR.

27. A peptide as defined in any one of Claims 1 to 26 in association with any suitable carrier and/or diluent and/or adjuvant.

28. A method of identifying a polypeptide suitable for use in the diagnosis of IM and related diseases, said method comprising:

(1) noting within EBV open reading frames transcribed late in the viral cycle for which a translation product may or may not have been established;

(2) synthesising one or more polypeptides each of which includes at least one segment wherein each segment comprises at least part of the amino acid sequence identified in that open reading frame; and

(3) determining whether said polypeptide is effective in the diagnosis of IM and related diseases.

29. A method of diagnosis of infectious mononucleosis or a related disease, said method comprising assaying serum from a patient suspected of having infectious mononucleosis or a related disease with at least one peptide as defined in any one of Claims 1 to 27.

30. A kit for use in the diagnosis of IM or a related disease, said kit comprising:

(a) at least one peptide as defined in any one of Claims 1 to 27; and

(b) a means for indicating the presence of an immunoreaction between said peptide and anti-EBV antibodies.

31. A kit for use in the diagnosis of IM or a related disease, said kit comprising:

(a) at least one peptide as defined in any one of Claims 1 to 27; and

(b) a means for indicating the presence of a reaction between said peptide and another molecule.

32. A vaccine that, when administered, is capable of inducing antibodies effective against infection by EBV, said vaccine comprising:

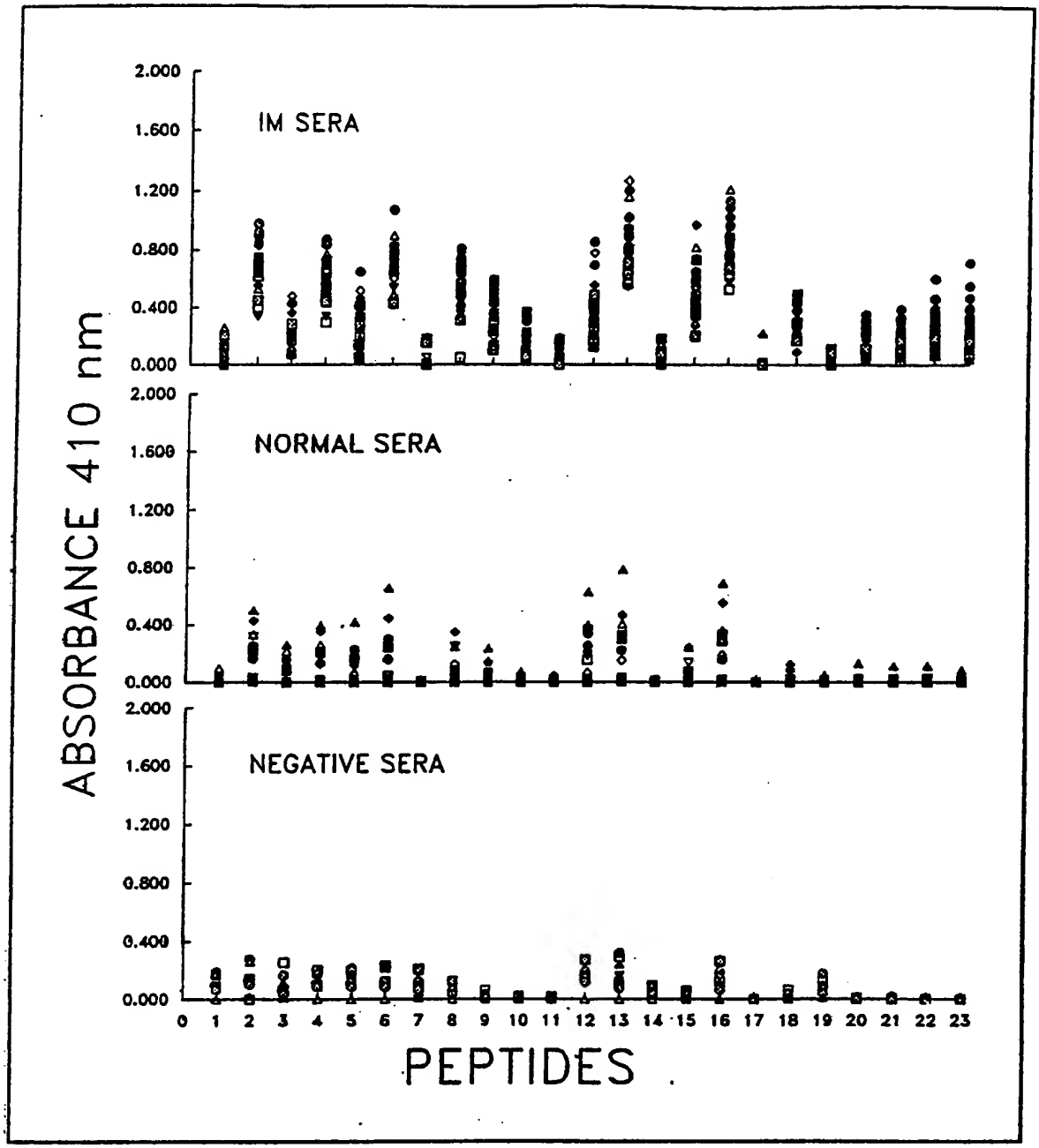
(a) at least one peptide as defined in any one of Claims 1 to 26; and

(b) a carrier and/or diluent and/or adjuvant.

33. Antibodies and substantially whole antibodies raised to - or induced by - at least one peptide as defined in any one of Claims 1 to 27.

34. A peptide as defined in Claim 1 substantially as described with reference to the Examples.

35. A method as defined in Claim 28 substantially as described with reference to the Examples.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NORMAL SERA
NEGATIVE SERA
ABSORBANCE 410 nm
PEPTIDES
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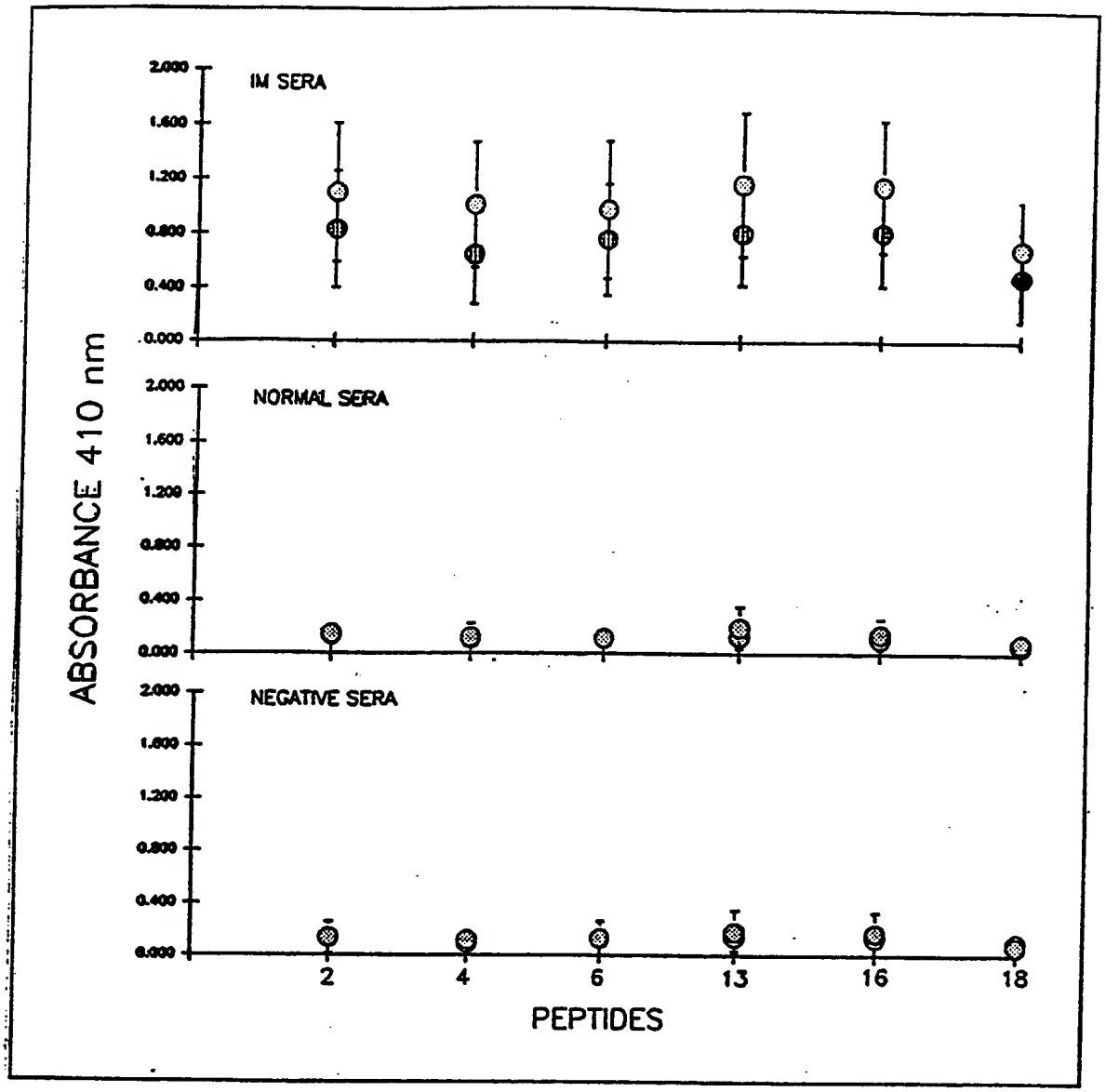
0.800
0.400
0.000
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

FIGURE 1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NORMAL SERA
NEGATIVE SERA
ABSORBANCE 410 nm
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1.600
1.200
0.800
0.400
0.000
2
4
6
13
16
18
PEPTIDES

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No. PCT/AU 90/00564

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[5] C07K 7/06, 7/08, 7/10, 15/12, C12N 7/00, C12Q 1/70, A61K 39/245, G01N 33/569

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
AU: IPC[5]	C07K 7/06, 7/08, 7/10, C07C 103/52, G01N 33/569

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

Chem. Abs. KEYWORDS: Epstein () Barr () Virus OR EBV and IM OR Infectious Mononucleosis. STN and PIR databases for Int. searching of seqs. of claims 13-20.

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category*	Citation of Document, [11] with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
X	AU,A, 82073/87 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 9 June 1988 (09.06.88) see pages 5,6 and claims	1 only
X,P	AU,A, 44000/89 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 22 February 1990 (22.02.90) see page 13 and claims	1 only
X	US 4707358 (KIEFF et al) 17 November 1987 (17.11.87) see example 4 and claims	1 only
X	Nature, Volume 310, issued 1984 July, (London) R. Baer et al, "DNA sequence and expression of the B95-8 Epstein-Barr virus genome" see whole document	1 only

(continued)

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27 February 1991 (27.02.91)	1 March 1991
International Searching Authority	Signature of Authorized Officer
Australian Patent Office	CEDRIC SCHAFFER

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Journal of Virological Methods, Volume 21, issued 1988 (The Netherlands) J. Middeldorp and P. Herbrink "Epstein-Barr virus specific marker molecules for early diagnosis of infectious mononucleosis", see whole document	1 only
X	Nucleic Acids Research, Volume 16 no.7, issued 1988 (IRL Press Limited, Oxford, England) D. Walls et al "The analysis of EBV proteins which are antigenic in vivo", see whole document	1 only
A	EP,A, 0316170 (G GENE GALWAY LIMITED) 17 May 1989 (17.05.89)	

V. [ ] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE 1

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.[ ] Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2.[ ] Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.[ ] Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4 (a):

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING 2

This International Searching Authority found multiple inventions in this international application as follows:

1.[ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.[ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.[ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 90/00564

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
US 4707358	EP 151079	JP 60232094	
EP 316170	JP 2002399		
AU 44000/89	WO 9001495		
AU 82073/87	DK 6388/87 JP 63225398 ZA 8709025	EP 280813 NZ 222794	IL 84690 US 4879213

END OF ANNEX